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Isolation of Plant Growth Promoting Rhizobacteria from *Spartina densiflora* and *Sarcocornia perennis* in San Antonio polluted salt marsh, Patagonian Argentina

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ABSTRACT

With the purpose of Plant Growth Promoting Rhizobacteria (PGPR) isolation, several authors have sampled different wetlands in the northern half of Argentina. However, to date, we do not know the existence of microbiological studies conducted in the Patagonian salt marshes, and concretely, concerning isolation of PGPR. The present work was conducted in the heavy metal polluted San Antonio salt marsh, in two areas with different grade of pollution. In those areas, we isolated cultivable bacteria from the rhizosphere of the halophytes *Spartina densiflora* and *Sarcocornia perennis*, and look for several plant growth promoting (PGP) properties among them. In total, 60 different cultivable bacteria were isolated. 50% of the rhizobacterial strains demonstrated at least one of the PGP properties assayed, 25% of them produced siderophores, 16% were able to solubilize phosphate, 11% were able to produce auxins and 7% chitinase. We could observe that PGP properties were more abundant among bacteria growing in polluted soils. Also, bacteria inhabiting *S. densiflora* rhizosphere showed more PGP properties related to heavy metal phytostabilization mechanisms, in line with the phytoremediation strategy of this halophyte. Overall, these findings support the idea that coastal hazardous scenarios may be a good opportunity to seek for PGPR. Indeed, some of the strains isolated in this work presented more than one PGP property, so they may be selected for further formulation of inoculants for different applications. For further research, it would be interesting to analyse other PGP properties in these strains, as well as to isolate rhizobacteria from other halophytes in diverse Patagonian salt marshes.

1. Introduction

Plants interact extensively with soil microorganisms, with reciprocal impacts on fitness. For example, Plant Growth Promoting Rhizobacteria (PGPR) are bacteria inhabiting the rhizosphere, in close contact with plant roots, able to enhance plant growth by a plethora of mechanisms (Glick, 2012). They have been studied for the last decades as potential agents to promote plant fitness under different biotic and abiotic stressors, such as salinity, drought, pathogens, pollution, etc. (Aeron et al., 2020; Beneduzi et al., 2012; Bhat et al., 2020; Guo et al., 2020). Many researchers aim to explore different ecosystems around the globe to isolate cultivable PGPR. In the end, the whole purpose is to propose bacterial candidates for multifunctional PGPR - based formulations, in order to use them with different applications. For example, commercial agriculture (to minimize the use of synthetic fertilizers and agrochemicals) (Backer et al., 2018), or as inoculants for phytoremediation (Thijs et al., 2017). Among PGPR, special attention has been paid onto salt-tolerant PGPR, as their use would be suitable in a wide range of saline lands (Arora et al., 2020; Sunita et al., 2020). A common place to isolate halotolerant PGPR are coastal wetlands, like salt marshes. They are flat drained areas of land which are subject to flooding by salt water and are usually covered by a mat of grassy salt-tolerant plants. These plants are known as halophytes, and in the last years, PGPR isolated from halophytes have emerged as a potential strategy in adaptive agriculture (Etesami and Beattie, 2018). This is especially important in the current scenario of climate change that we are going through, where salinity of soils is increasing (IPCC, 2014). Salt tolerance and capacity of ameliorating plant fitness of halotolerant PGPR make them ideal

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biofertilizers in saline soils or under situations of irrigation with saline water (Mesa-Marín et al., 2019a).

San Antonio salt marsh is located in the northeastern Patagonia, in Río Negro province, Argentina, and it provides important ecosystem services, like coastal protection and site for resting, feeding and nesting of many birds and seabird species (Martinetto et al., 2010). Also, it contains a considerable amounts of heavy metals. Surrounding this salt marsh, there are deposits of the mining wastes originated by the metallurgical extraction of polymetallic sulphide that were deposited more than four decades ago in the proximities of San Antonio Oeste City (Idaszkin et al, 2015, 2017). It is a situation common to other salt-marshes, due to their natural position, between marine and terrestrial environments, which makes them historically sites of human colonization, commerce and industry development. Urbanization of these areas have motivated burning, filling and draining of wetlands, control of water resources, and discharge of wastes into waterways, which explains worldwide estuarine vulnerability to heavy metal inputs (Catallo, 1993; Ridgway and Shimmield, 2002; Williams et al., 1994). Anyhow, this situation means that PGPR isolated from polluted estuarine areas may be not only salt-tolerant, but also heavy metal-tolerant, making them ideal candidates to be used as potential heavy metal bioremediators (alone or in combination with phytoremediator plants) or as biofertilizers in environments contaminated with heavy metals (Colin et al., 2012). For example, authors of this work have isolated heavy metal- and salt-tolerant PGPR from different halophytes (*Spartina densiflora, Spartina maritima, Arthrocnemum macrostachyum, Halimione portulacoides* and *Salicornia ramosissima*) growing in heavy metal polluted salt marshes in Spain (Andrades-Moreno et al., 2014; Mesa-Marín et al., 2019b; Mesa et al., 2015a; Navarro-Torre et al., 2016). Some of those PGPR have been studied as potential inoculants under greenhouse conditions, demonstrating increases in plant growth, photosynthetic fitness and metal accumulation or stabilization (Mesa-Marín et al., 2018, 2020a, 2020b, 2020a; Mesa et al., 2015c). Also, they promoted germination improvements in bacterized seeds that were subjected to a salt gradient (Mesa-Marín et al., 2019b).

With the purpose of PGPR isolation, several authors sampled different wetlands in the northern half of Argentina, like the Salado River Basin (Castagno et al., 2011), the Salí River in the Río Hondo reservoir (Amoroso et al., 1998), or the rhizosphere of halophytes like *Prosopis strombulifera* in El Berbedero saline (Sgroy et al., 2009) or *Sesuvium portulacastrum* from Santiago del Estero (Lami et al., 2020). However, to date, we have not known the existence of microbiological studies conducted in the Patagonian salt marshes, and concretely, concerning isolation of PGPR, which opens a new opportunity of research. In the past years, authors of this work carried out functional studies in San Antonio salt marsh, which reported the pollution levels in soil and the role that vegetation played in the biogeochemical cycle of some metals (Idaszkin et al, 2015, 2017). Thus, today we know that there is a pollution gradient, which goes from the area of the salt marsh closest to the mining wastes (more concentrated) to the entrance of the tidal channel (more diluted) (Idaszkin et al., 2015). Also, special attention has been paid during these studies on the dominant halophytes inhabiting San Antonio salt marsh, *Spartina densiflora* and *Sarcocornia perennis* (Bortolus et al., 2009; Isacch et al., 2006). It was observed that both species were able to grow in metal polluted soils and to accumulate in their tissues metals like lead, zinc and copper (Idaszkin et al., 2017).

Therefore, the present work aims to isolate cultivable bacteria from the rhizosphere of *Spartina densiflora* and *Sarcocornia perennis* in areas of San Antonio salt marsh with different grade of pollution, and look for several PGP properties among them. It is a descriptive work that constitutes the first screening of PGPR in the Patagonian Argentina and another contribution to the study of PGPR strains isolated from natural ecosystems, in order to discover potential bacterial candidates for biofertilizer formulations.

2. Materials and methods

2.1. Sampling sites description

The present study was conducted in San Antonio salt marsh (40°44′S, 54°68′W), a Natural Protected Area in Río Negro, Patagonian Argentina (Fig. 1). Two sampling sites were selected within the salt marsh, adjacent to the main tidal channel (Fig. 1). Site A is in the head of the channel and it is highly polluted. It is very close to a large open-air dump that has been operational for over three decades and, due to the topography, this is also a zone largely influenced by the mine superficial run-off erosion (Idaszkin et al, 2015, 2017). Site B is less polluted, as it is located in the outer zone of the channel (Idaszkin et al, 2015, 2017). In both sites, within the high salt marsh level, co-inhabit the halophytes *Spartina densiflora* and *Sarcocornia perennis*. Idazskin et al. (2017) studied physicochemical properties of soil in both sampling sites of this study. Site A had an electrical conductivity of 7.68 mmhos cm^{-1}, 5.84% organic matter, pH 7.61, a redox potential of 139.6 mV, % of clay/silt/sand 22.63/55.57/21.79, Cu content 38.72 μg g^{-1}, Fe 14.23 mg g^{-1}, Pb 63.9 μg g^{-1} and Zn 221.6 μg g^{-1}. Site B had an electrical conductivity of 3.97 mmhos cm^{-1}, 3.08% organic matter, pH 7.65, a redox potential of 167 mV, percentage of clay/silt/sand of 3.85/27.41/68.74, Cu content 4.98 μg g^{-1}, Fe 12.99 mg g^{-1}, Pb 7.66 μg g^{-1}, and Zn 17.2 μg g^{-1} (Idaszkin et al., 2017).

2.2. Isolation of cultivable bacteria from Spartina densiflora and Sarcocornia perennis rhizosphere

In August 2019, samples of rhizospheric soil of three randomly selected *Spartina densiflora* and three *Sarcocornia perennis* were collected from sites A and B (Fig. 1). Two rhizospheric soil samples from each plant were collected using a stainless steel soil core sampler to a depth of 20 cm, in two different points next to the plant stem. The mentioned replicates (2 cores × 3 plants x 2 species x 2 sites = 24 samples) were collected in order to get more representative samples of the rhizosphere. The samples were placed in individual plastic bags, transported to the laboratory and stored at 4 °C. The day immediately after, rhizobacteria were isolated. For that, soil loosely adhered to roots was removed and the samples of each rhizosphere soil (four in total; *S. densiflora* rhizosphere from A and B, and *S. perennis* rhizosphere from A and B) were thoroughly mixed. Then, 1 g of each pool, containing roots and firmly adhered soil particles, were placed in four separated 50 mL sterile flasks and mixed with 40 mL of physiological saline solution (NaCl 0.9% w/v), by shaking for 15 min using a rotary shaker. After that, big particles and sediments were allowed to settle for 10 min. Then, 100 μL of the supernatant suspensions, 10^{-1} and 10^{-2} dilutions were plated onto tryptic soy agar (TSA) medium plates. Following the incubation for 48 h at 28 °C, single colonies were selected according to differences in colony morphology and subsequently re-isolated again by plating on TSA and incubated at 28 °C for 48 h in order to obtain pure cultures. Gram staining was used to describe bacterial morphology and cell wall, as well as to check purity of strains. Purified bacterial cultures were preserved in 15% glycerol at −80 °C for further use. Medium selected was TSA because it is a general medium that would permit the isolation of bacteria without special requirements, which may facilitate screening tests *in vitro* and further optimization and implementation of bacterial bioinoculants.

2.3. Screening for plant growth promoting traits in isolated rhizobacteria

Plant growth promoting traits were analyzed at the Institute of Biotechnology and Molecular Biology (STAN 3041, National University of La Plata, National Scientific and Technical Research Council, Argentina). Phosphate solubilization, as well as production of siderophores, auxins and chitinase were determined as explained in López et al. (2018). Briefly, the ability to solubilize mineral phosphate was

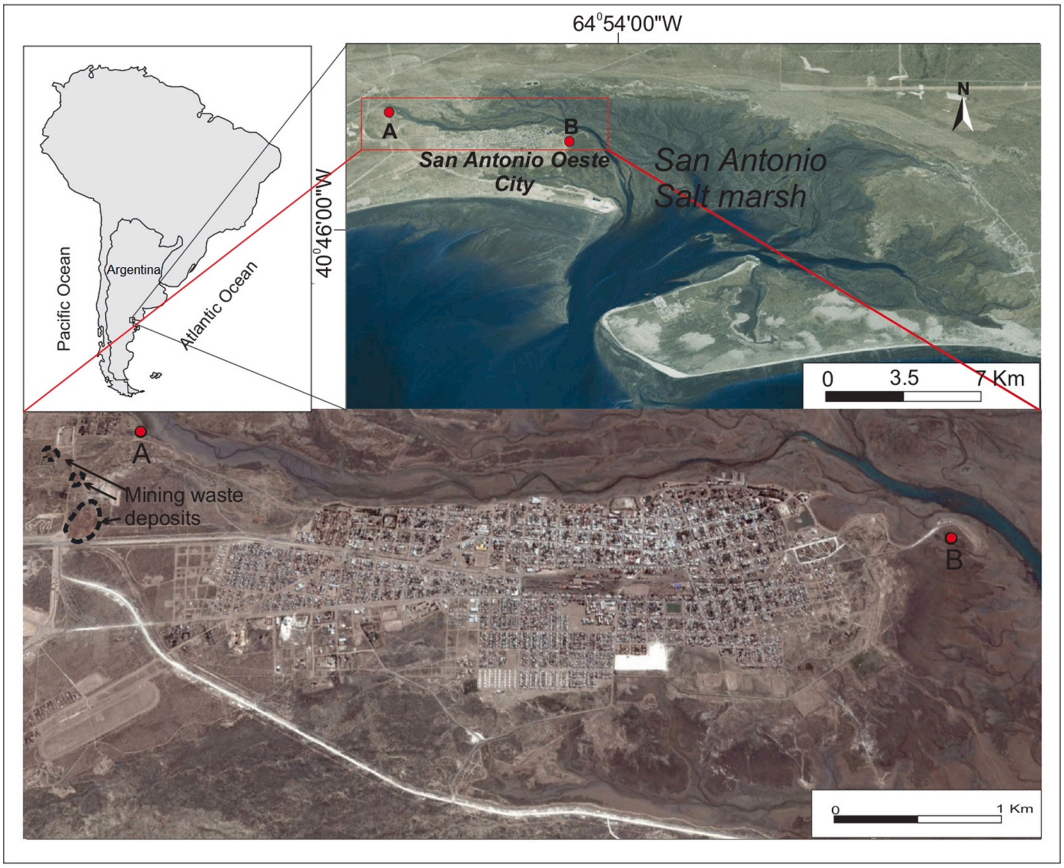

Fig. 1. Main geomorphological units and location of the sampling sites A and B in the San Antonio salt marsh, Rio Negro, Argentina (Idaszkin et al, 2015, 2017).

detected by formation of transparent halos around bacterial colonies on plates containing National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999). Siderophores production was screened in chrome-azurol-S agar plates (Louden et al., 2011). Isolates were considered to be siderophore-producing when an orange halo of diameter greater than 5 mm was formed around the bacterial colony after 5 days of incubation at 28 °C. The ability to produce auxins was determined by quantification of indole-3-acetic acid (IAA) production. It was analyzed from 4-day-old bacterial cultures grown in LB broth supplemented with 500 μg mL^{-1} of the acid's precursors tryptophan in the dark at 30 °C, with Salkowski's reagent (Patten and Glick, 2002). The amount of IAA produced was expressed as μg IAA per mg protein. Total protein content of each isolate was determined by Bradford assay (Bradford, 1976). A strain was considered IAA producer when it was able to produce a minimum of 10 μg IAA mg^{-1} protein. Finally, to test the chitinase activity, bacterial isolates were grown on solid medium with colloidal chitin prepared as indicated by Shimahara and Takiguchi (1988). The colloid-containing plates contained 1.5 g chitin, 2.7 g K_2HPO_4, 0.7 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.5 g KCl, 0.13 g yeast extract, 15 g agar in 1 L distilled water. Chitinase production was confirmed by the generation of a clear halo around a colony after 10 days of growth at 28 °C.

3. Results

In total, 60 different cultivable bacteria were isolated from the two halophytes growing in sites A and B in San Antonio salt marsh, 29 from the rhizosphere of *S. densiflora* and 31 from the rhizosphere of *S. perennis*.

Globally, just over half of the total rhizobacterial isolates tested (51.8%) demonstrated at least one out of the four plant growth promoting (PGP) properties assayed (Table 1). 24.1% of them produced siderophores, 16.6% were able to solubilize phosphate, 12.9% were able to produce a minimum of 10 μg IAA per mg protein and 7.4% produced chitinase. None of the 60 rhizobacteria showed the four PGP properties studied. Strain DC8 was the only strain with three activities out of the four assayed.

Fig. 2 schematically presents, at a glance, global results and results grouped by sampling site and halophyte. In the case of rhizobacteria isolated from *S. densiflora* in both sites, the 29 isolates were rod-shaped and 62.1% of them were Gram negative (Table 1). More than half of the isolates (57.7%) had at least one out of the four PGP properties assayed. 34.6% of them produced siderophores, 19.2% solubilized phosphate, 11.5% produced more than 10 μg IAA mg^{-1} protein and 15.4% produced chitinase. Concerning bacteria isolated from *S. perennis* rhizosphere in A and B sites, 68% of the 31 isolates were Gram negative, 87% were rod-shaped and 13% coccus (Table 1). Somewhat less than half (42.9%) had at least one PGP property of the four tested. In equal proportion, 14.3% of the isolates produced siderophores, solubilized phosphates and produced IAA, while any strain from the rhizosphere of *S. perennis* produced chitinase. Finally, it is also interesting to consider the results grouped per areas. From the 60 bacterial strains, 35 were isolated from more polluted site A and 25 from less polluted B. The most outstanding result was that 57.6% of rhizobacteria from polluted soil A presented at least one PGP property, while this data dropped to 38.1% in those rhizobacteria from less polluted area B. Siderophores production was more widespread amongst bacteria from highly polluted soil (18.3% in site A versus 3.3% in site B); solubilization of phosphates followed a similar, but no so accused, trend with 10% of solubilizers in A and 5% in B; and finally, production of IAA and chitinase production were equally represented in both sites, by 5% of strains in each area in the case of IAA production and 3.3% for chitinase producers.

Table 1
Characteristics of the sixty bacterial isolates from *Spartina densiflora* and *Sarcocornia perennis* rhizosphere, growing in (A) polluted and (B) non-polluted areas from San Antonio salt marsh (Río Negro, Argentina). For each strain, the table shows bacterial shape, rod (R) or coccus (C), Gram staining (+or -), absence (+) or presence (−) of activity for siderophores and chitinase production and phosphates solubilization; and production of auxins (indole-3-acetic acid, IAA) in μg IAA mg^{-1} protein (positive production was considered when values resulted over 10 μg IAA mg^{-1} protein). Shaded cells highlight positive results. Strains DA18, SA17, DC2, DC7, SC11 and SC13 did not maintain viability during PGP tests, so they were not determined (nd).

Location	Hosting plant	Strain	Shape	Gram	PGP activities			
					Siderophores	Phosphates	IAA	Chitinase
A	*Spartina densiflora*	DA1	R	–	–	–	2,63	–
		DA2	R	+	–	+	4,08	–
		DA3	R	+	++	–	3,62	–
		DA4	R	+	++	+	1,42	–
		DA5	R	+	–	–	2,92	–
		DA6	R	+	+	–	3,87	–
		DA7	R	–	++	–	5,32	+
		DA8	R	+	++	–	4,58	–
		DA9	R	–	++	–	4,74	+
		DA10	R	+	–	–	3,98	–
		DA11	R	+	–	–	2,76	–
		DA14	R	–	+	–	3,1	–
		DA17	R	–	–	–	8,37	–
		DA18	R	–	nd	nd	nd	nd
		DA19	R	+	–	+	3,92	–
		DA20	R	+	–	–	1,25	–
		DA22	R	–	–	–	12,49	–
	Sarcocornia perennis	SA1	C	+	–	+++	5,48	–
		SA2	R	–	–	–	1,86	–
		SA3	C	+	+	–	2,06	–
		SA4	R	+	+	–	6,71	–
		SA5	R	–	+	–	2,58	–
		SA6	R	–	–	–	1,42	–
		SA7	R	–	–	–	2,45	–
		SA8	R	+	–	+	2,21	–
		SA9	R	–	–	–	12,32	–
		SA10	R	–	–	–	1,65	–
		SA11	C	+	+	–	1,82	–
		SA12	R	+	–	–	4,02	–
		SA13	R	–	–	–	1,63	–
		SA14	R	–	–	–	10,08	–
		SA15	R	+	–	+	2,78	–
		SA16	R	–	–	–	1,87	–
		SA17	R	–	nd	nd	nd	nd
		SA18	R	–	–	–	2,31	–
B	*Spartina densiflora*	DC1	R	–	–	–	9,96	–
		DC2	R	–	nd	nd	nd	nd
		DC3	R	–	–	–	3,52	+
		DC5	R	+	++	–	2,89	–
		DC6	R	–	–	–	12,05	–
		DC7	R	–	nd	nd	nd	nd
		DC8	R	–	++	+	6,25	+
		DC9	R	–	–	–	3,47	–
		DC10	R	–	–	–	1,45	–
		DC11	R	–	–	+	3,83	–
		DC12	R	–	–	–	1,69	–
		DC13	R	–	–	–	6,16	–
	Sarcocornia perennis	SC1	R	–	–	–	1,24	–
		SC2	C	+	–	–	2,86	–
		SC3	R	–	–	–	1,65	–
		SC4	R	+	–	+	2,98	–
		SC5	R	–	–	–	2,17	–
		SC6	R	–	–	–	12,13	–
		SC7	R	–	–	–	1,29	–
		SC8	R	–	–	–	10,23	–
		SC9	R	–	–	–	5,21	–
		SC10	R	–	–	–	1,42	–
		SC11	R	–	nd	nd	nd	nd
		SC12	R	+	–	–	2,14	–
		SC13	R	–	nd	nd	nd	nd

4. Discussion

In this study we isolated, for the first time, cultivable bacteria from a salt marsh in Patagonian Argentina and studied some PGP *in vitro* abilities. In particular, we isolated rhizobacteria from two perennial halophytes cohabiting in the San Antonio Bay in Río Negro (Argentina), these are, the austral cordgrass *Spartina densiflora* and the pickleweed *Sarcocornia perennis*.

The first observation that came out was that Gram positive rhizobacteria were dominant in polluted areas of the salt marsh. This fact has been also observed in different salt marshes in Spain by authors of this work and other colleagues (Andrades-Moreno et al., 2014; Mesa et al.,

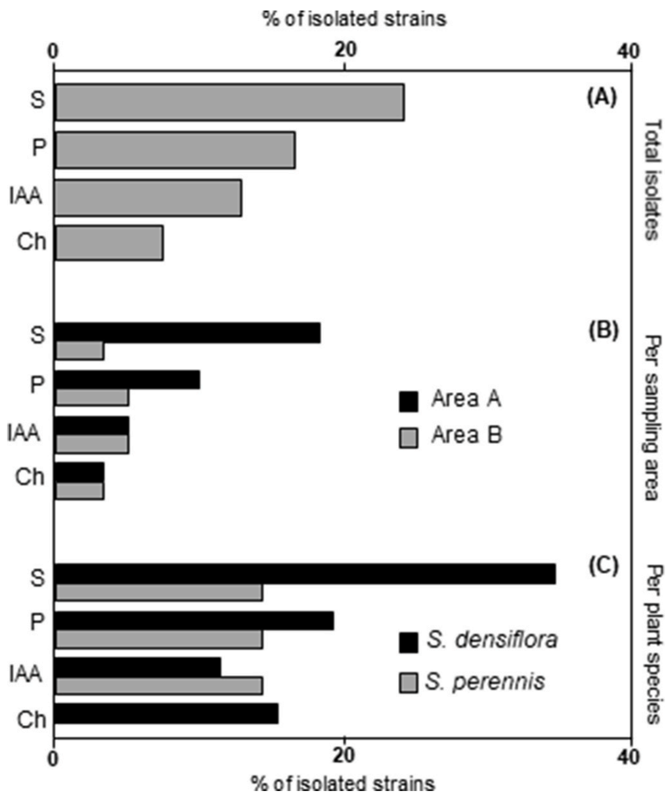

Fig. 2. Plant growth promoting (PGP) properties found in rhizobacteria isolated in this study, expressed as relative data. S, siderophores production; P, phosphate solubilization; IAA, auxins production; Ch, chitinase production. (A) Represents the PGP properties found among all the viable isolates, while (B) shows the PGP properties in rhizobacteria classified by sampling area of origin (more polluted A and less polluted B) and (C) by hosting plant species (*Spartina densiflora* rhizosphere and *Sarcocornia perennis* rhizosphere).

2015a; Paredes-Páliz et al., 2016a), as well as in Argentina, like in "El Bebedero" saline (Sgroy et al., 2009). Gram positive bacteria have structural characteristics that confer more resistance to stressful conditions like pollution (Nies and Silver, 2007), so it may explain the fact that they are commonly present in hazardous scenarios (Roane and Kellogg, 1996).

Another remarkable conclusion of our study was that PGP properties were more abundant among rhizobacteria established in the polluted area of San Antonio salt marsh (area A) rather than in the non-polluted one (area B). Specifically, production of siderophores was notably more present. From the data, it could be stated that iron content in both sites was quite similar and it did not reflect an iron deficit (Idaszkin et al., 2015). Then, the rationale behind this fact may be the presence of heavy metals, as siderophores production is especially important for iron availability in the presence of overwhelming amounts of other potentially competing metals (Glick, 2010). Besides, previous work has shown that the great presence of copper and nickel in San Antonio salt marshes (Idaszkin et al., 2015) may compromise iron mobilization (Schenkeveld et al., 2014). Together with siderophores production, phosphate solubilization was also more frequent in bacteria from polluted rhizosphere. In this sense, several researchers have found that bacteria that facilitate phytoremediation sometimes have an active phosphate solubilization system, which may play a role in assisting metal uptake (Glick, 2010). This is in accordance with the observations of Idaszkin et al. (2017), who considered *S. densiflora* and *S. perennis* in San Antonio saltmarsh as candidates for phytostabilization of heavy metals due to their capacity to phytostabilize metals like Pb, Zn and Cu. Another observation they did in their study was that heavy metals were more accumulated in below-ground structures when they grew in soils with high metal concentrations. However, in soils with low metal concentrations these species were able to translocate more heavy metals to above-ground tissues. Along with our results, this fact supports the hypothesis that PGPR may be promoting phytostabilization of heavy metals in high polluted soils, through abilities such as siderophores production or phosphate solubilization, as also observed other authors (Mateos-Naranjo et al., 2015; Mesa et al., 2015c; Paredes-Páliz et al., 2017).

What is more, Idaszkin et al. (2017) proved that *S. densiflora* and *S. perennis* showed different metal phytoaccumulation patterns. They concluded that *S. perennis* translocated more metals to the aerial structures than *S. densiflora*, which means that *S. densiflora* stabilizes more heavy metals in its roots. When we observed the data of PGP in rhizobacteria per hosting plant, we observed that siderophores production and phosphate solubilization were more present among rhizobacteria isolated from *S. densiflora*, which again reinforces that PGPR may be directly involved in processes that aid heavy metal solubilization, bioavailability and mobility for plant root phytostabilization. For that, they may use mechanisms like metal chelating with siderophores, metal complexation by production of low molecular weight organic acids, synthesis of biosurfactants that desorb metals from the soil matrix, or promotion of chemical reactions like oxidation, reduction or biomethylation, among others (Ullah et al., 2015).

This descriptive work supports the idea that coastal hazardous scenarios may be a good opportunity to seek for plant growth promoting bacteria, as reported previously in other polluted salt marshes (Mesa et al., 2015a,b; Navarro-Torre et al., 2016; Paredes-Páliz et al., 2016a,b), or demonstrated by authors working in extreme environments (Yadav, 2017). Then, it would be of interest to isolate rhizobacteria from other halophytes in diverse unexplored Patagonian salt marshes. For example, in our study strain DC8 was able to produce siderophores and chitinase, to solubilize phosphates, and had an auxins production that, although it was not considered positive for our study, it was still higher than the average. Then, this strain may be selected for further formulation of inoculants. For that purpose, it would be useful to deeper characterise strains by analysing other PGP properties in these strains, as nitrogen fixation, biofilm formation, ACC deaminase production or K solubilization, as well as salinity and heavy metal tolerance. Finally, conclusions reached in this study should be interpreted cautiously, as our aim was to isolate PGPR and we discussed possibilities that explain higher proportion of isolates with a specific ability in one location or plant species. To analyse the influence of pollution or plant species on the presence of PGPR, a deeper study would be required, with different culture media, in more locations, complementary techniques, etc.

5. Conclusion

In the today's world there is a need to enhance crop production and soil fertilization, but also climate change is leading to increased salinity of soil and irrigation water. In this scenario, the knowledge about halotolerant bacteria associated to rhizosphere is relevant (Mesa-Marín et al., 2019a; Sáenz-Mata et al., 2016). They may have potential applications as biofertilizers in adaptive agriculture and enhancing the salinity tolerance of non-halophytic crops. On the other hand, they may also be used for heavy metal phytoremediation purposes in soils with high salinity (Liang et al., 2017; Litalien and Zeeb, 2020), or as a source of new enzymes and metabolites (Mesa-Marín et al., 2019a). In our work, we isolated and characterized rhizobacteria from an unexplored salt marsh till date, in the Patagonian Argentina. Plant growth promoting properties, specially production of siderophores and solubilization of phosphates, were more abundant among bacteria growing in polluted soils. Also, bacteria inhabiting *S. densiflora* rhizosphere showed more plant growth promoting properties related to heavy metal phytostabilization mechanisms, in line with the phytoremediation strategy of this halophyte.

Credit author statement

Yanina L. Idaszkin: Resources, Funding acquisition, Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Rosana Polifroni: Methodology, Writing – original draft, Writing – review & editing, Jennifer Mesa-Marín: Resources, Funding acquisition, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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